Original Paper

# PRP Enriched with Hyaluronic Acid -PRP from Rat Protocol and Method of Preparation-

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**ABSTRACT:** Observing the positive effects of PRP (platelet-rich plasma) used in various pathologies, both in traumatology, orthopedics, sports medicine, and in plastic and reconstructive surgery, we decided to develop an improved product, using granular hyaluronic acid. The paper aims at establishing a protocol for obtaining PRP enriched with hyaluronic acid, which can be used in the current practice of treatment of skin defects, safely, with minimal side effects and limited possible, but to provide a shorter healing period as compared to native, "free" healing. The experiment aims to find an effective and rapid method of healing wounds with skin defects, by using a local adjuvant (PRP enriched with hyaluronic acid), which is available to any plastic surgeon. Following the combination of PRP with granular hyaluronic acid, we obtained a product that macroscopically has a gelatinous, viscous consistency, with a good adhesion to the tissues. The potential benefits of this experiment could be the basis for the development of treatment protocols for various pathologies, which result in wounds with skin defects, the most important aspect being the shortening of the classic healing period.

***KEYWORDS:*** *Platelet-rich plasma, hyaluronic acid, rat, protocol, preparation.*

## Introduction

PRP or platelet-rich plasma is defined as an autologous product, which contains a higher platelet concentration as compared to the baseline of 150.000-350.000 platelets/µl within whole blood [1,2].

Platelets play a very important role in tissue regeneration and are involved in re-vascularization, hemostasis and the construction of new connective tissues [1,2].

Platelet growth factors are released slowly and thus promote the healing processes that occur at the site of PRP application.

In recent years, special attention has been paid to the utilisation and continuous improvement of this "wonder product", being used in various medical fields [3,4], such as orthopedics [2,5-7], rheumatology, urology [8], dermatology, oral-maxillo-facial surgery, aesthetic surgery and last but not least, plastic surgery.

In order to comply with medical ethics and the rigorous of science, any experimental use of PRP should be performed first in a controlled laboratory, using Wistar rats, most commonly used as experimental subjects [5,6,9,10].

The rat is a good candidate for scientific and experimental research, as it has many advantages, such as: the ease it can be procured, handled, stored, fed and monitored.

These advantages propel him to the forefront of research for healing in various tissues, so that the rat is perhaps the most widely used laboratory animal. Being a versatile laboratory animal, the rat becomes one of the species used in various experiments such as osteo-integrable different materials area or stroke research.

The most used methods of collecting blood from rats are represented by: intracardiac transthoracic collection [6,10], collection from the sinus of the retro-orbicular plexus and blood collection from the jugular vein [11].

Observing the positive effects of PRP use in various pathologies [12-14,15,16] like in traumatology, orthopedics, sports medicine, reproductive disease [14], pain therapy [17] and in plastic and reconstructive surgery, we decide to develop an improved product, using granular hyaluronic acid.

The paper aims at establishing a protocol for obtaining PRP enriched with hyaluronic acid, which can be safely used in the current practice of treatment of skin defects, with minimal side effects and limited costs, but to provide a shorter healing period compared to native "free" healing.

## Material and Method

The experiment took place at the Experimental Research Facility of the University of Medicine and Pharmacy of Craiova, with the agreement of the Ethics Commission and complied to all the rules of animal protection.





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The experiment aims to find an efficient and fast method of healing wounds with skin defects, by using a local adjuvant.

The potential benefits of this experiment could be the basis for the development of treatment protocols for various pathologies, which result in skin defects.

The instruments used were the classic surgical ones. We used syringes with sodium citrate of 3.8%, the collection being made on a peripheral venous catheter of 26G.

The experimental batch consisted of living Wistar laboratory rats. The substance used was granular hyaluronic acid in powder form. General anesthesia with ketamine and xylazine was performed.

The experimental group consisted of 30 individuals (Wistar laboratory rats) (Figure 1), clinically healthy, males and females, aged between 3 and 6 months and weighing between 350 and 450 grams, these being divided into 3 sublots of 10 individuals each, which are sacrificed at 7, 14 and 21 days.

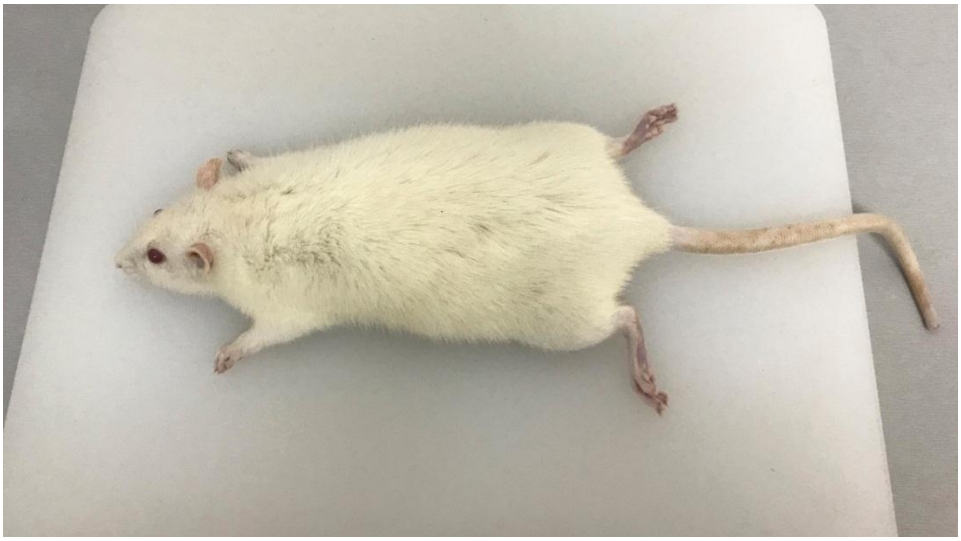

***Figure 1. Wistar laboratory rat.***

## Results

The general anesthesia was used. Induction was performed using the ketamine/xylazine combination, at a dose of 90mg/10mg/kg administering a single subcutaneous injection.

The first operative step was to identify the jugular vein for blood collection.

The cleaning of the skin at the level of the ventral chest wall was performed with aseptic solution and an incision of about 2cm was made on the midline.

During dissection, the jugular vein was identified at about 1 cm from the midline, on the left side (Figure 2).

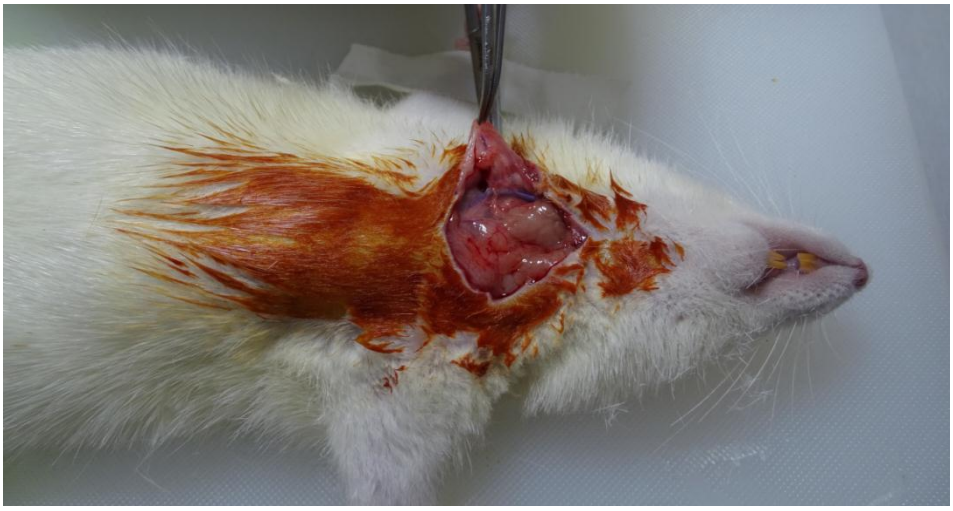

***Figure 2. Jugular vein identified at about 1cm from the midline, on the left side.***

The jugular vein was punctured and a 26G peripheral catheter was inserted and a sample of 4ml is extracted (Figure 3).

The collected blood was immediately replaced with sterile saline in the same amount (4ml).

The catheter was removed and hemostasis by local compression was performed.

After checking the hemostasis, lavage was performed with antiseptic solutions (betadine) and skin was sutured.

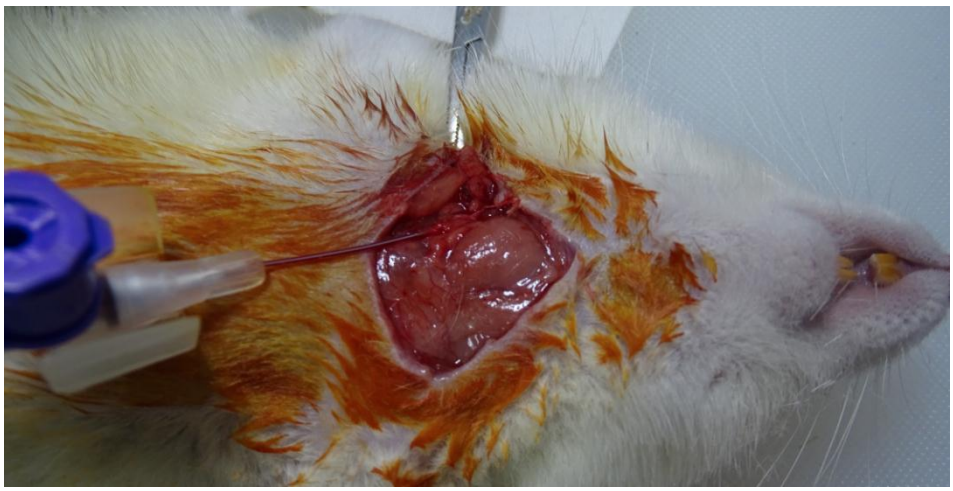

***Figure 3. 26G peripheral catheter inserted into the jugular vein.***

The second operative step was the thorough disinfection of the skin at the level of the dorsal chest wall.

There have been two skin defects, on an individual basis, of about 1cm² each, at a distance of about 2cm each, using a scalpel blade number 10/11.

The defect was made strictly at the level of the skin, without exceeding this area.

Hemostasis control and lavage with antiseptic solutions were practiced (Figure 4).

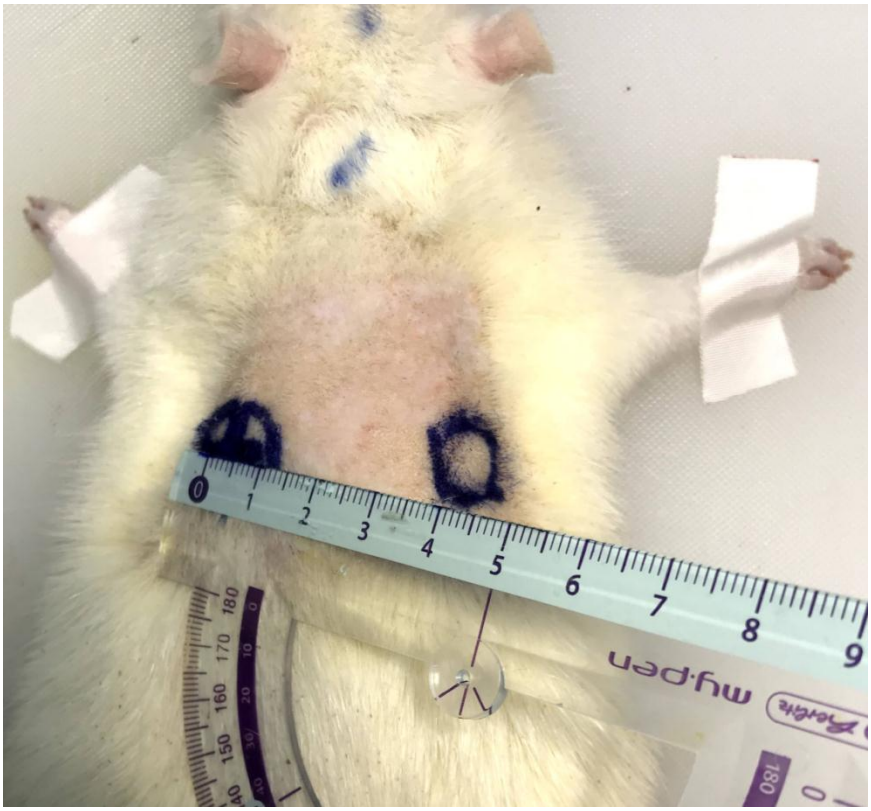


***Figure 4. Skin defects preoperative drawing.***


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The defect made on the left side was used as the control site, which was left for natural healing.

The defect on the right side was the working variable, at the level of which PRP enriched with hyaluronic acid was applied.

Over the defects was applied a dressing type hydrofilm.

Blood for PRP was collected on a 26G peripheral catheter in a syringe with 0.4ml of 3.8% sodium citrate.

The amount of approximately 4.5ml obtained was transferred to a 5ml test tube.

The contents were slightly homogenized, after which it was centrifuged for 15 minutes at 1500 rpm (Figure 5).

***Figure 5. The content centrifuged for 15 minutes at 1500 rpm.***

Plasma was carefully extracted without introducing red blood cells into the syringe, resulting in approximately 2ml PRP (Figure 6 and Figure 7).

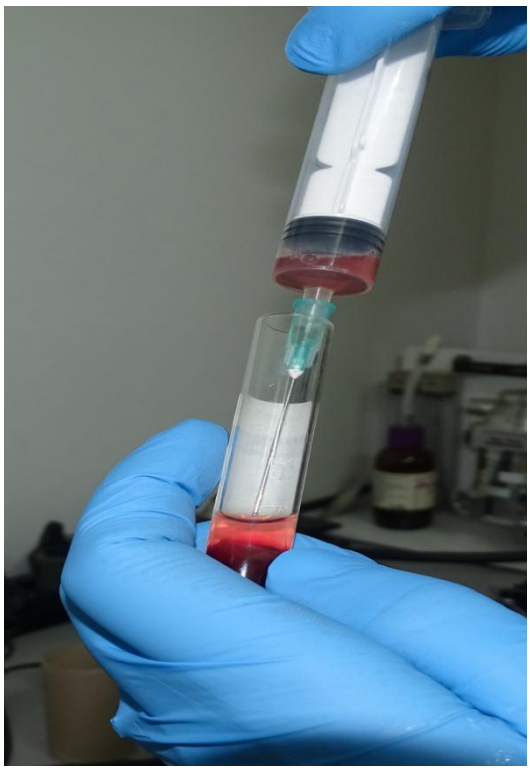

***Figure 6. PRP extraction.***

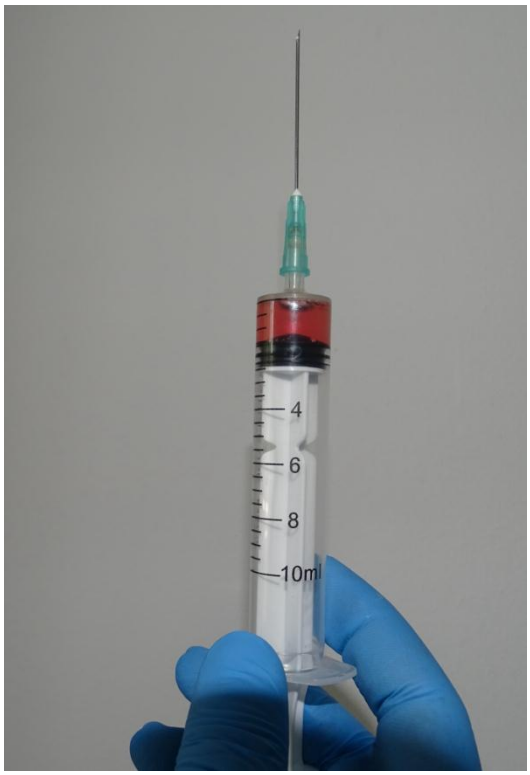


***Figure 7. The final result-2 ml PRP.***

Platelet-rich plasma was activated with calcium gluconate of 96mg/ml, in a ratio of 1:10.

It was enriched with hyaluronic acid, so that to 1ml of activated PRP is added 25mg of

hyaluronic acid powder, with a molecular weight of 10-25kDa.

Hyaluronic acid was incorporated into PRP by light, circular movements, until a homogeneous mass with a gelatinous, adherent consistency was obtained after approximatively 2 minutes of mixing. (Figure 8 and Figure 9)

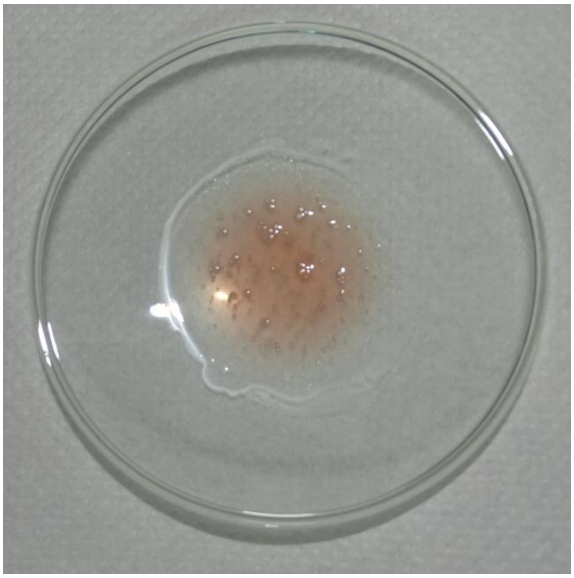

***Figure 8. The product obtained from the incorporation of the hyaluronic acid into PRP.***

***Figure 9. The consistency of the PRP enriched with hyaluronic acid.***

Each individual in the group was given antibiotics before and after surgery, in order to reduce the risk of postoperative infections.

It was used a 40mg/kg/day dose of Cefoperazone (1000mg) / Sulbactam (1000mg).

Also, after the intervention, Tramadol 20mg/kg/day was administered, intramuscularly, to reduce the discomfort caused by defects.

The individuals were followed by the resulting wounds, at 7, 14, and 21 days, respectively, with careful monitoring of possible signs of local infection.

Tracking the evolution of wounds is performed both macroscopically and microscopically, and immunohistochemically.

## Discussions

In the context of animal experiments, a moderate demand for them was estimated, with a minimum to moderate level of suffering.

There should be no adverse effects following the local application of PRP enriched with hyaluronic acid.

However, there are risks associated with the individual response to the anesthetic protocol used and postoperative infections.

Postoperative complications were minimized by following the surgical protocol, checking the hemostasis, by observing asepsis and antisepsis, as well as by adapting the doses of the substances used according to each individual.

A controlled environment in terms of comfort and hygiene were ensured, so that neither the wounds nor the health of individuals are compromised.

The individuals of the experimental group were accommodated in accordance with the provisions of Law no. 43/2014, being provided fresh water and fodder ad libidum.

Their handling was performed by qualified personnel, and the administration of the substances were done in accordance with good practices in the field.

At the end of the experiment the animals used were slaughtered.

From the 2ml of PRP resulting from the centrifugation we obtained a sufficient amount of product to be used in 5 individuals per group, so that harvests were required from 2 individuals each.

The consistency of the product enriched with hyaluronic acid was the same after each harvest and preparation, without undergoing major macroscopic changes, and its application was easy, without complications.

The purpose of this experiment is to find a method that is cheap, easy to obtain, but effective in potentiating the healing of wounds with skin defects.

We decided to follow and observe the evolution of wounds treated with PRP enriched


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with hyaluronic acid, as we believe that if each product used as such had good results in this pathology, combined should potentiate each other, thus leading to much better results in a shorter time, and with high quality scars.

We also looked for a method of blood collection that is easy, handy and does not lead to the slaughter of an animal in addition to the experimental group.

## Conclusions

The six individuals used to collect blood from the jugular vein survived and were used in the experimental group.

This led to the minimization of animals slaughtered in the experiment.

The collection of blood from the jugular vein was relatively easy, the only impediment being the approach of the vein to smaller individuals, so it was decided to use the largest individuals in the group for collection.

They provided the desired amount of blood without postoperative complications.

Also, a very important factor in harvesting from the jugular vein is the size of the peripheral venous catheter used, so that a catheter larger than 26G will irreparably damages the vein.

The incorporation of the hyaluronic acid powder in PRP was done gradually, and the homogenisation of the product was done manually, by light, circular movements.

Thus, a homogeneous product was obtained, with a gelatinous, adherent, slightly elastic consistency.

The colour of the product was influenced by the initial colour of the PRP.

The application of pears on enriched with hyaluronic acid was done at the level of the working variable so as to cover the wound entirely in a generous layer, at which time the adhesion of the product to the underlying tissues was highlighted.

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This article has not been funded by any public or private institution.

This article is not advertised for any commercial product, so the trade names of any product used in the experiment were not used.

Our intention is not to facilitate the sale of any of the products used in the experiment, its purpose being to find a method of rapid healing and within the reach of any plastic surgeon.

### Conflict of interests

None to declare.

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