

**UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA  
DOCTORAL SCHOOL**

**Doctoral Thesis**

**Abstract**

**PLATELET CONCENTRATE AUGMENTED BONE HEALING**

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## I. INTRODUCTION

Tissue healing and inflammatory response management are a matter of great concern to physicians around the world. The particular case of bone healing and the different management issues in regard to regulating bone healing and conducting it to the desired golden standard of *restitutio ad integrum* are a particular branch of research that sits at the intersection of cell biology, clinical orthopaedics and immunohistochemistry. Due to the high impact that a fracture can deal in an individual's wellbeing and its social implications, we consider that all matters of research that can aid the healing of a fracture should be at the forefront of research in any serious Research Centre.

Platelet concentrates are a new addition to the arsenal of clinical aids at the disposition of physicians in the race against inflammation and in order to promote tissue healing. They have been used in areas of plastic surgery, sports medicine, orthopaedic surgery, oral and maxillofacial surgery and dentistry.

Due to the relative novelty of platelet concentrates used in the field of bone healing further studies are needed in order to be able to clearly state when where and how these should be used.

We are performing an experimental study in order to assess the benefits of using a new technique of Platelet Rich Fibrin at the site of a bone defect.

## II. THE CURRENT STAGE OF KNOWLEDGE

### Chapter 1. Fractures

Fractures, in general, represent one of the most common traumatic injuries to the musculoskeletal system, with up to 41,25% cases that have presented to the Emergency Department of The Clinical Emergency Hospital Bucharest over a period of 47 years [1]. The high socio-economic impact that a fracture has on the patients life drives research worldwide to enhance the body's own healing and thus reduce healing time.

Normal fracture healing occurs, from a histological point of view, either by primary healing or secondary healing [2,3].

Primary bone healing involves the crossing of the fracture gaps by bony cutting cones thus generating cortical remodelling. This means of healing is achieved in special circumstances,

which involve an anatomical reduction of the fracture site, rigid internal fixation combined with compression of the fracture fragments, circumstances that occur only in case of open surgical fracture reduction and a rigid internal fixation [4].

Secondary bone healing involves endochondral and intramembranous ossification that occur while micro-motions are present at the fracture site.

These local phenomena occur in three phases [5]. The first phase involves blood that flows from the fractured bone which develops into the primary hematoma. This hematoma consequently generates a local inflammatory response with the release of macrophages that include interleukins, neutrophils, platelets and cytokines that include platelet derived growth factor (PDGF) [6]. Fibroblasts and mesenchymal stem cells (MSC) migrate at the fracture site with the aid of cytokines. It was first believed that MCS migrate from the local environment of exposed bone marrow and surrounding soft tissues. Recent data shows that MCS are recruited from the circulating reservoir of MCS [7,8]. The osteoblasts and fibroblasts multiply locally and granulation tissue is formed around the fracture fragment ends. The primary hematoma develops into a fibrin rich granulation tissue [9]. The osteoblasts and fibroblasts multiply locally and granulation tissue is formed around the fracture fragment ends. The primary hematoma develops into a fibrin rich granulation tissue [10]. The following phase is the reparative phase, when primary callus formation occurs. The fibrin rich granulation tissue acts as a matrix for soft callus made up out of cartilaginous tissue. Laboratory studies show that, in rats, soft callus is formed after 7-9 days following the traumatic bone incident [11]. Intramembranous ossification occurs at the same time, in the sub periosteal area of the bone, adjacent to the distal and proximal ends of the fractured bone thus generating a hard callus. The bridge that eventually forms between the two fractured ends represents a semi-rigid structure that allows weight bearing. The third phase represents bone remodelling according to Wolff's Law of response to bone stress [12].

## Chapter 2. The use of platelet concentrates in tissue healing

Due to the high degree of limitations and contraindications of pharmaceutical aid of tissue healing [13] researchers have been trying to develop biologically compatible aids from platelet concentrates. Platelet concentrates are extracted from a specimen of whole blood taken from the patient that is processed through various techniques, usually by centrifugation [14]. This is

performed in order to separate by gradient weight and keep only the blood elements that are potentially useful for tissue healing such as platelets, fibrin (fibrinogen) and leukocytes and discard the less useful elements such as red blood cells. This process is aided by the fact that red blood cells are much heavier and thus easily separated.

The fact that platelet concentrates are tissues belonging to the individual themselves assures complete biocompatibility.

The first step of the natural wound healing process is the formation of a platelet and fibrin clot. Upon this realization, the next logical step was to try to increase the local concentration of platelets in an effort to supplement the natural healing process.

The precursor of platelet concentrates were fibrin glues that have been first used more than 40 years ago [15,16]. An evolution of this idea was proposed in 1979 with the use of platelet gel [17], a mixture that is based on a high concentration of platelets in the final product.

Platelet rich fibrin (PRF) was developed in France [18] and was different enough from all previous platelet concentrates that the authors were compelled to name it a “second generation platelet concentrate”. What set PRF apart from all previous platelet concentrates was the strong fibrin polymerization that occurred around the concentrated platelets which gave it plastic characteristics that were useful in particular clinical scenarios.

### Chapter 3. Rat experimental research on bone healing

The literature [19] describes producing intraosseous defects in rats and observing the healing rate as the best scientific method of determining the effects of different materials and biomaterials that are studied for the purposes of bone healing. There have been different models of bony defects that are available for the bone healing researcher. These include long bone defects, the defect of a segment of the mandible or, as this thesis is studying, calvaria defects.

### Chapter 4. Cone-Beam Computer Tomography

Cone-beam Computerized Tomography (CB-CT) is a relatively new radiological image procurement method that is founded upon a cone shaped model X-ray beam that projects onto a

plane receiving surface. The X-ray emitting source and the plane receiving the X rays perform a full circumrotation around the investigating object producing an array of two dimensional images along the way. One of the reasons for choosing CB-CT over conventional  $\mu$ CT was because of the ALARA principle (“As Low As Reasonably Achievable” pertaining to the radiation dose received by the investigated subject<sup>[20]</sup>) of the International Commission on Radiological Protection (ICRP). Rat calvaria defects are evaluated by CB-CT by applying the Patel score [21].

### III. OWN CONTRIBUTIONS TO THIS TOPIC

#### Chapter 1. Thesis objectives

The goals of this study consisted of trying to have a better understanding of the impact that an autologous Platelet Rich Fibrin implant has in fracture/bone defect healing in a standardized setting that consisted of 3 mm sized defects that were performed in rat calvaria. In order to investigate this issue to its full extent the distinct aims of this research were set according to these guidelines:

1. To evaluate the inherent regenerative capacity of rat calvaria bone to heal the 2.5 mm diameter defect without any outside influences;
2. Providing the same bench frame for the Platelet Rich Fibrin enhanced defect in order to have reliable outcomes. Any advantage in healing rates/bone quality developed in the PRF enhanced defect can only be obtained from the supposed benefits of PRF;
3. To provide with an easy protocol for obtaining rat PRF that can be easily and consistently reproduced
4. All of our animal studies have been performed with the accept of the Animal Welfare Commission of the University of Pharmacy and Medicine of Craiova

## Chapter 2. Materials and Methods

We used 35 male Wistar rats supplied by the Bio Base of the University of Pharmacy and Medicine Craiova. The average weight of the rats was 350 g, ranging from 250-420g. The rats were chosen to be at least 6 months of age, in accordance with the guidelines for rat calvaria research.

The rats were housed in individual cages for the duration of the experiment, held in temperature controlled, air-conditioned rooms, with controlled lighting. Water and food pellets were provided *ad libitum*.

Sterile working conditions were assured for the duration of the surgical procedures.

The specific requirements of our study necessitated for the establishment of particular protocols for obtaining PRF from rats as well as a technique for inducing the calvaria defects.

The relatively large quantity of blood required for PRF preparation (10 ml of blood required vs 12-15 ml of total blood in a rat [22]) lead us to use cardiac puncture as the technique of choice for obtaining the necessary blood. This resulted in a donor rat supplying the blood required for the preparation of 4-5 PRF grafts. The donor PRF grafts were usable between rats as Wistar lab rats lack specific antigenicity.

The base centrifugation protocol for obtaining PRF was the Choukroun method.

We induced two defects in the rat calvaria by using a 3 mm trephine and augmented the right defect with PRF.

Histological and radiological assessment of bone defect healing was performed at 45 days post-intervention.

The method used for radiological assessment of calvaria defect healing was by using the scoring system of rat calvaria defect bony bridging and union of Patel adapted for CB-CT scanning.

## Chapter 3. Results

Surgical results – improvements upon the established surgical technique

The standard technique for opening the periosteum for inducing two defects is performing a longitudinal incision, periosteum stripping and suturing it. This allows for mobilisation of the periosteum flaps, greatly increasing the risk of graft mobilisation upon suturing, as well as establishing a communication pathway between the two defects, thus decreasing the accuracy of the results.

The surgical technique was improved by inducing two consecutive periosteal incisions, first dealing with the left-control defect and then with the right-test. This greatly decreased the risk of graft mobilisation upon suture involved with regular single sagittal incision of the periosteum.

Upon the left-control defect induction, by using a 3 mm trephine, the periosteum was sutured on top of it, effectively restoring periosteum integrity. The right incision was consequently performed, and the right-test defect was induced with the same trephine. The previously prepared PRF graft was cut to size and introduced in the defect. The periosteum was sutured on top of it. This technique allowed for minimal periosteum stripping and thus as little as possible periosteum mobilisation when suturing, as well as secluding the two defects, in order to obtain accurate results.

#### Quantification of the platelet concentration in the PRF clot

We devised a innovative method [23] for quantifying the platelet count and platelet concentration from a non-liquid PRF clot. The technique involves producing PRP from a equivalent blood sample. Our measurements determined the Platelet concentration of 4,155,553/mm<sup>3</sup>. When compared to the platelet concentration of whole blood we observed an increase in platelet concentration of 489% in the PRF clot.

#### Cone-Beam Computer Tomography results

Each rat involved in the experiment was assessed by CBCT at 45 days after the defect induction. The Patel calvaria defect healing score of each defect was determined. After image procurement the experimental aspect of the thesis was considered finished and the animals were sacrificed.

The left-control defect had the following healing scores:

- 10 defects had a score of 0;
- 18 defects had a score of 1;
- 5 defects had a score of 2;
- 2 defects had a score of 3&4.

The right-test defect had the following healing scores:

- 3 defects had a score of 0;
- 6 defects had a score of 1;
- 17 defects had a score of 2;
- 9 defects had a score of 3&4.

The results clearly show a favorable effect incurred by the addition of PRF in the right-test defect, as outlined by the differences in the scores showing the greatest amount of bone healing, “Score 2” and “Score 3&4”, 5 vs. 17 and 9 vs. 2, left-control vs. right-test defects respectively.

#### Statistical results

We applied a statistical calculation to the data contained using the t-test score and the subsequent value of  $p$ .

The analysis of the data resulted from the CB-CT investigation generated a t-test value of -4.19921 and a  $p$  value of .0004.

We have thus proven the statistical relevance of the hypothesis, with a threshold set at  $p < 0.001$ , generating a degree of certainty of 99.9 % that the healing that had occurred in the right test defect was generated by the addition of PRF.

The statistical calculations were performed by using specialized statistical software.

Also, the magnitude statistical value of  $d$  – Cohen [24] was calculated at 1.002692. by using Coe’s conversion table [25], we are able to state that the magnitude of the effect that PRF had on the healing of the rat calvaria defects was 84 %.

#### Histological results

When compared to the “right-test” defect, the “left-control” defect showed signs of delayed bone formation with either no bone densification occurring in the conjunctive tissue filling the defect or singular bone formation, with no outgrowths typical of an ongoing healing process. Thus, we concluded that the left-control defect did not show any of the rapid healing process occurring in the right-test defect.

## IV. DISCUSSIONS

PRF augmentation has the ability to increase the natural healing capacity of the bone. The only issue that stems from usage of PRF in a clinical scenario is represented by the increased risk of infection, brought on by the removal of the blood sample from the sterile surgical field and the subsequent manipulation of the PRF graft after the centrifugation process. Great care needs to be taken to decrease the risk of infection.

A very important aspect of choosing the rat as the experimental animal [26] consisted of the possibility to reliably induce two identical calvaria defects in the same animal [27]. This gave us the possibility to have identical starting conditions for the two defects. The left defect was the control and the right was the test PRF addition defect. The fact that we could induce a self-comparing defect within the same individual allowed for a great reduction in the total number required for the experiment as well as increasing the reliability of the results.

## V. CONCLUSIONS

Our study has successfully proven that the addition of PRF to a bone defect enhances the body's natural healing possibilities.

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