УДК 615.46

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PREPARATION OF PLATELET-RICH PLASMA: EXISTING METHODS, PROBLEMS OF STANDARDIZATION

Platelet rich plasma (PRP) is a component of blood with concentrations of platelets above normal values. Scientific discussions about manufacture protocols and equipment for PRP derivation have still been holding. However, the design of conducted researches, a group of patients, evaluation of clinical efficiency, the use of methods, machines for manufacturing are distinguished in many ways. All existing research results are difficult to interpret and sort out, as there are many protocols for commercial and manual methods for PRP preparation.
The aim of this review was to identify the most appropriate and reasonable method of PRP preparation – we took into account optimal speed and centrifugation time, anticoagulant for inhibiting platelet aggregation process and an activator for the growth factors release.
Conclusion: Commonly double centrifugation is used to obtain PRP. The variety of speed and time of centrifugation depends on the centrifuge model and its force. The therapeutic effect of the use of PRPis achieved by increasing the concentration of platelets by different sources 2-8 times, on average 5 times, depending on the initial content of platelets in blood. In studies often we observed 10 minutes centrifugation and speed not less than 160 g, but not more than 3000 g. As an anticoagulant, the use of sodium citrate was more common.The calcium chloride solution and autologous thrombin were used as an activator of PRP in most studies.
***Keywords**: Platelet rich plasma preparation, production.*

Introduction.
The main goal of modern surgery is to optimize and accelerate the regeneration of tissues. In recent years it has been presented as a concept of regenerative surgery. Many other techniques have been widely described in the literature. The most commonly used methods of regenerative surgery are: the usage of PRP (platelet rich plasma), PRGF (plasma rich with growth factors) and PRF (fibrin-rich plasma) techniques [1].
PRP (platelet rich plasma) is plasma, that has been enriched with platelets [2].The separation of blood into its components for future usage in surgery has a long history. In 1998, Marx reported about the effectiveness of platelet-rich gel usage for the regeneration of mandible defects [3]. Since platelet-rich plasma has been used in various fields of surgery to the healing of soft tissue wounds in aesthetic surgery, treatment of burns, nerve pathology, chronic ulcers.
Scientific discussions about production protocols and equipment to obtain platelet-rich plasma are conducted until now. However, the design of the fulfilled researches, a group of patients, evaluation of the clinical efficiency, the usage of methods, machines for producing are different in many ways [5],which explains the lack of standardized methods for the preparation and application of autologous platelet-rich plasma [6, 7].
All existing research results are difficult to interpret and sort out, as there are many protocols for commercial and manual methods for platelet-rich plasma preparation. Effects provided by platelet-rich plasma, depend on the methods of its preparation as one technique enables to stimulate the proliferation and differentiation of cells ultimately, and others conversely leads to a lack of results.
Purpose of review: identification of the most appropriate and reasonable method of platelet rich plasma preparation – speed and centrifugation time, anticoagulant for inhibiting platelet aggregation process and an activator for the growth factors release.
Materials and methods:
Search in databases: MEDLINE Complete, eBook Clinical Collection (EBSCOhost), Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews, Cochrane Methodology Register, eBook Collection (EBSCOhost).Patented methods of obtaining of platelet-rich plasma in the territory of the CIS are also viewed.
Keywords: Platelet rich plasma preparation, Platelet rich plasma production, Platelet rich plasma obtaining, PRP preparation.
Inclusion criteria for searching in databases: full-text original articles; articles, written not earlier than in 2000; language of articles is English;
Exclusion criteria: abstracts, articles repetition; studies before 2000.
Analyzed data: a method of obtaining platelet-rich plasma, anticoagulant types, used for the preparation of platelet rich plasma, speed and centrifugation time, the use of commercial systems, the rationale for choosing a particular speed and time of centrifugation, the use and justification of one or another platelet activator.
Results and discussion:
Since 1975, according to the search results, keywords, 117 works has been found, after setting the time frame from 2000 and later - 91 work. With the exclusion of abstracts and full-text repeats the original article in volume of 24 has been found.
Arshdeep, M.SendhilKumaran (2014) in his work emphasized that platelet rich plasma can be prepared manually or by using automatic devices. For the inhibition of platelet aggregation process, the plasma preparation is carried out with the addition of anticoagulants, citrate dextrose (ACD-A) or sodium citrate. Platelets should be isolated in high concentrations to achieve a therapeutic effect and growth factors secretion.The author describes the double centrifugation technique, recalling that in the technical guideline of the American Association of Blood Banks.PRP is separated from blood by “light-spin”centrifugation, and platelet concentration is a result of its “heavy-spin” centrifugation.The scheme for obtaining platelet rich plasma includes: an assemblage of 40-75 ml of blood via venipuncture into tubes with 15 ml of an anticoagulant (ACD-A 10: 1.5) by centrifugation at a force of 160xg for 10 minutes at 20° C, whereby blood is separated into plasma, “buffy coat” - a layer containing platelets, leukocytes and erythrocytes. The further plasma and "buffy coat" assemblage and centrifugation is conducted at a force of 400xg for 10 minutes at 20° C [7].
In 2012, Merolla M. et al.studied the effect of the centrifugal force onaggregometry. Although, the investigation doesn’t refer to the study of platelet rich plasma obtaining for therapeutic purposes, their results demonstrate the change in the number and average volume of platelets depending on the centrifugation force. In this work, centrifugation of 10 control samples at a force of 150, 200, 300 and 500xg during 10 minuteswas carried out. It was noted that the reduction of platelets number and the decrease in the average volume of platelets are in direct proportion with the speed of centrifugation.Here are the results of changes in the platelet numbers, depending on speed: 50 g - 464.5 [399 536.3], 200 g - 441.5 [382, 540], 300 g - 375.5 [338.8, 453.3], 500 g - 257.5 [187.8, 345]; and changes in the average platelet volume: 150 g - 8.1 fl [7.2, 9.0], 200 g - 7.8 fl [7.1, 8.8], 300 g - 7.7 fl [6.8, 8.5], 500 g - 6.7 fl [6.1, 7.5] [8].
Breddin H. (2006) in the article discussed various anticoagulants for preparation of platelet rich plasma and finally offered recommendations for time and speed of centrifugation.A frequently used anticoagulant is sodium citrate dihydratein a concentration of 3.13% or 0.106 mol/L. Another alternative is hirudin. Blood with the addition ofhirudin contains a normal concentration of calcium and magnesium. Hirudin is used in a concentration of 5 mg/ml - 0,1 ml per 10 ml of blood. It is also possible to use heparin and other thrombin inhibitors. The author notes that many researchers adjust the number of platelets in the plasma using a mix of rich and platelet-poor plasma to achieve a platelet amount varied between 200 and 300 000 per µl. The mixing process leads to platelet activation, and we must remember that the aggregation response does not change in the range of platelet number from 150 000 to 450 000.Recommendations of author: to obtain platelet-rich plasma anticoagulated blood is centrifuged at room temperature at a force of 500xg within 4 minutes. At higher force or at long centrifugation time, most platelet areget lost [9].

Angad Malhotra et al. (2013) also described the preparation of platelet rich plasma, its properties and application. Preparation begins with blood harvesting and mixing with an anticoagulant. Sodium citrate or acid citrate dextrose-A (ACD-A) may be used as anticoagulants, which are most widely used for preparation of platelet-rich plasma [4]. ACD-A is capable to support mechanisms of signal transduction in the PRP preparation process and therefore to maintain of platelets responsiveness to activation [10].There isalso information that EDTA (ethylenediaminetetraacetic acid) minimizes platelet aggregation more effectively, but traditionally it is recommended not to use it because of the potential of irreversible changes in the structure, biochemistry and functions of platelets [11].Also, there are well-known methods ofplateletpheresis[12] and filtration [13] at the preparation of platelet-rich plasma.

The authors presented a method of production of platelet rich plasma by the result of double centrifugation, the first hard spin centrifugation separates erythrocytes from the plasma, and the second soft spin separates leukocytes from the platelets as described with reference to the author Marx R. [2].

Very often the commercial systems for preparation of platelet-rich plasma uses a single centrifugation, the aim of which is separation of the blood into three layers - erythrocytes, leukocytes and plasma directly. Thus, except leukocytes, the middle layer contains platelets, and is often taken together with the upper layer of plasma, which is called platelet poor plasma. However, depending on the parameters of centrifugation and techniques, this layer may contain a large number of platelets [14]. Positive sides of commercial systems usage instead of manual are due to the stability and ergonomic than the platelet concentration efficiency.

The authors also focus on the fact that the literature presents a variety of options of single and double spin centrifugation, its force, speed and rotation time. In the clinical studies described optimal force and time (160-3000xg within 3-20 min), which didn't lead to the loss of quality of platelets [15]. In this interpretation of the term of platelet-rich plasma as a portion of autologous blood platelet concentrations above average does not give a clear definition of optimal amount of platelets in prepared plasma. Many authors are still defined by Marx R., whereby platelet rich plasma - a product with a concentration $1000x10^9$/L in 5 ml of plasma or 1,000,000/µl. The average value of platelets in the blood equal to $200x10^9$/L in (150 - 350 thousand/µl). Studies show that an increase in concentration in 2-8 times, in average 5 times, has a therapeutic effect [2].

Araki et al. (2012) compared results of the different forcesduring single and double centrifugation.Authors reached a maximum - 20 times increase of the content of platelets in plasma by double centrifugation at a force of 230xgfor 10 minutes at the first centrifugation, and at 2300xg for 10 minutes at the second centrifugation. As an anticoagulant, the preference was given to EDTA. The ultimate goal of this study was to achieve the highest concentration of platelets in the plasma, while the therapeutic effects of platelet-rich plasma were inconsistent [16].

Dugrillonet al. (2002) reported a decrease in the content of TGF-β growth factor by centrifugation at force more than 800xg within 15 min [17].

Weibrichet al. (2003) examined the therapeutic effects of platelet-rich plasma prepared in various ways and found that the concentration of platelets in whole blood exceeds the content of 2 and 6 times the most desired because at higher concentrations results of treatment were absent. The authors explained it by the fact that the potential growth factors is the transduction of cellular response that is limited by the expression of the associated receptors on targeted cells, so the excessive increase of growth factors may lead to no results [18].

The method of platelet-rich plasma activation is not standardized in practice. There are two opinions about the need to activate the plasma. According to the first opinion, platelet-rich plasma is activated in the case of direct introduction of it into the tissue physiologically. In this case, the main task of platelet rich plasma practical usage is to enter plasma into anticoagulated condition into damaged tissues, taking into the consideration the fact that platelet-rich plasma is activated in vivo in contact with collagen. Regarding the activation of ex vivo, bovine thrombin was the most suitable activator, but caused complications [19]. Therefore, a number of other author opposed the usage of bovine thrombin to activate the platelet-rich plasma. For example, according Lais Fernanda Marques et al. (2015), platelet-rich plasma is plasma with platelet concentrations greater than in whole blood, which is achieved by multiple centrifugation. Before its usage in the practice, plasma must be activated for the platelet growth factors release. The usage of bovine thrombin can cause coagulopathy due to the production of antibodies against coagulation factors. To the opinion of the authors, an alternative method is to use collagen or autologous thrombin. The immediate introduction of platelet-rich plasma into tissue without pre-activation is a tempting alternative because activation can occur while injecting, - at soft tissues trauma, with a needle [20].

A more convenient and widely used in practice activator is a solution of calcium chloride [21]. Other agonists such as ADP, thrombin and collagen interact with surface receptors, triggering the intracellular signal addressed to platelet granules [22]. Nowadays, an autologous thrombin extracted from the patient's own blood to activate the prearranged autologous plasma is also used.

Lorenzo Dragoet al. (2012) conducted a study, taking the blood from 20 healthy donors.Blood collected intubes with 3.8% trisodium citrate as anticoagulant. Blood was centrifuged at a force of 580xg for 8 min at room temperature.Threecomponents were obtained - erythrocytes, leukocytes, and plasma. Further, the plasma fraction located above the red cell fraction was collected and centrifuged according authors scheme (3000 g for 10 min). Activation of the PRPcarriedout before the usage with a solution of calcium chloride [23].

Kellie K.Middletonet al. compared eleven different commercial systems for the preparation of platelet-rich plasma in their article. Five of them are recommended to use the protocol with double centrifugation. Authors described the required volume of blood collection, the amount of the resulting platelet-rich plasma and platelet concentration in it, the need to add an activator and the activator type. The most criticized aspect according to the authors for obtaining of rich plasma is a force. The force in excess of 800xg leads to loss of platelet granules and reduceamount of growth factors releasing [24].

Paola RominoAmableet al. (2013) studied various speed variations of time and temperature during centrifugation for platelet rich plasma preparation to determine the optimal conditions in which platelets do not lose their properties. The blood of 22 healthy volunteers, blood sampling was carried out in vacuum tubes with citrate. Plasma preparation included a double centrifugation. In the first centrifugation empirically applied various force, time and temperature. To optimize the process of preparation of platelet rich plasma in the second centrifugation empirically studied variation of force and time. In the experiment marked the three most suitable conditions (temperature 12 ° C): 700 g force, time of 17 minutes; 450 g force, time of 12 minutes; 800 g force, time of 12 minutes [25].

Diogo Franco et al. (2012), reproduced the following protocol: 40 ml blood sampling into the tubes containing 3.2% of sodium citrate. Centrifugation at a force of 400xg for 10 min. Further was carried a removal of portion obtained platelet-poor plasma, sampling and separation of layer, containing platelets and leukocytes (buffy coat) into two tubes. The plasma in the first test tube was used for the production of platelet-rich plasma, the plasma in the second tube - for thrombin. Only 1.5 ml of plasma was used for the production of thrombin, where 10% of calcium gluconate was added and incubated for 15 minutes at 37 degrees. Then, the two tubes were centrifuged again at a force of 800xg within 10 min. Then platelet-rich plasma was obtained into the first tube and thrombin-rich plasma into the second. Further the removal of 2/3 of the volume of plasma from the first tube (platelet poor plasma) was carried out, the remaining 1/3 of the volume contained platelet-rich plasma [26].

Alsousou J. et al. (2013) in their article noted that traditionally the obtain of platelet-rich plasma in laboratories is carried out under the following conditions: the force of 170-200xg, centrifugation time - 10 minutes at room temperature. But for the use of blood products in surgery there are no standardized methods, there are three variants of obtaining platelet-rich plasma: the gravitational sequestration, standard separation andplateletpheresis. The authors primarily examined commercial systems in their work by which it is possible to obtainplatelet rich plasma [27].

Double centrifugation is widely used to obtain platelet-rich plasma. A variety of speed and centrifugation time depending on the model of centrifuge and its centrifugal force. Nevertheless, in a series of researches parameters are obtained empirically. The therapeutic effect of the

platelet-rich plasma is achieved by increasing the concentration of platelets in the different sources in 2-8 times (on average in 5 times) depending on the original content of platelets in the whole blood. In most cases we observed figuring time of 10 minutes and the force is not less than 160xg, but not higher than 3000xg. As an anticoagulant are increasingly common used the sodium citrate, as activator in addition to calcium chloride encountered repeated references to the feasibility of using autologous thrombin. However, an alternative option is a use of a single centrifugation method applying commercial systems, easy-to-use and patented. In this case, all the parameters for platelet-rich plasma preparation were obtained empirically and confirmed experimentally for a particular commercial system.

Conclusions:

Undoubtedly, today there is a variety of protocols to obtain platelet rich plasma and there is no single standardized method. After analysis, we scanned the information and came to the following conclusions about the parameters in the course of preparation of platelet-rich plasma.

1. Multiplicityof centrifugation: double centrifugation is often usedfor obtainingplatelet rich plasma, herewith the data about characteristics ofthe first and the second centrifugationare different (what it should be hard or soft spin); single centrifugationis mainly used in commercial systems.
2. Platelet concentration: the platelet count should be greater than the initial level of platelets in whole bloodin 2-8 times (average in 5) for therapeutic effect.
3. Force or speed of centrifugation:contradictory results, but clearly force should benot below 160xg and not more than 3000xg.
4. Used anticoagulants: sodium citrate, dextrose, EDTA, hirudin, heparin.
5. Used activators: calcium chloride, bovine thrombin, autologous thrombin. It is also possible a physiological activation in tissue after injection of the plasma, especially when using as anticoagulant sodium heparinate which does not bind calciumas citrate.

A variety of methods is also associated with a variety of used equipment, different in different countries, requiring certain parameter settings for force and centrifugation time to achieve therapeutic effect. That is why the best in our opinion, is an independent experimentally achievement of a certain force and time of centrifugation to obtain platelet-rich plasma, or, in case of failure of the experiment the use of patented, certified commercial instructions, which give clear guidance on the use of certain parameters.

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ТРОМБОЦИТКЕ БАЙ САРЫ СУДЫ ДАЙЫНДАУДЫҢ БАР ӘДІСТЕРІ ЖӘНЕ ЖҮЙЛЕСТІРУ МӘСЕЛЕЛЕРІ

Түйін:Тромбоцитке байсарысу (PRP) - бұл, құрамында тромбоциттер концентрациясы қалпынан жоғары сарысу. Тромбоцитке байсарысуды алу құралдары және салыстырмалы дайындау жолдары жайында ғылыми таластар әлі күнге дейін жүруде. Алайда орындалған зерттеулер суреттемесі, науқастар топтары, клиникалық тиімділік бағасы, қолдану әдісі, дайындау құралдары бойынша едәуір айырмашылық тарыбар. Зерттеу қортындыларын іріктеу және түсіндіру күрделілеу, өйткені тромбоцитке бай сарысуды саудалық және мануалдық тәсілдер үшін дайындау жөнінде көптеген хаттамалар бар.
Осы шолудың мақсаты, неғұрлым тиімді және дәлелденген тромбоцитке бай сарысуды дайындау тәсілін, айналым жылдамдығын және уақытын, тромбоциттің агрегациялық процессін тежеу үшін антикоагуляторды және өсу факторларын босататын белсендірушіні анықтау болып табылады.
Қортынды: Тромбоцитке бай сарысуды алу үшін көпшілік қосайналымды қолданады. Жылдамдық айырмашылығы және айналым уақыты центрифуганың моделіне және айналым күшіне байланысты. Тромбоцитке бай сарысуды қолданудың емдік тиімділігі кейбір деректемелер бойынша тромбоциттің концентрациясын 2- 8 рет жоғарылатқанда жететінін, толық қанның алғашқы құрамында орта шамамен 5 ретке дейін болуына байланысты. Біз көбіне уақыттың 10 мин және жылдамдықтың 160 g. Кем емес, бірақ 3000 g. жоғары болмауын бақыладық. Антикоагулянт ретінде көбіне натрий цитратының қолданылатынын, белсендіруші ретінде кальций хлорид ертіндісімен бірге аутологиялық тромбинді қолданудың тиімділігі жайында бірнеше ескертуді кездестірдік.
Түйінді сөздер: тромбоцитке бай плазма, алу, дайындау

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ПРИГОТОВЛЕНИЕ БОГАТОЙ ТРОМБОЦИТАМИ ПЛАЗМЫ: СУЩЕСТВУЮЩИЕ МЕТОДЫ, ПРОБЛЕМЫ СТАНДАРТИЗАЦИИ

Резюме: Обогащенная тромбоцитами плазма (PRP) – это плазма, концентрация тромбоцитов в которой превышает нормальную. До сих пор ведутся научные дискуссии относительно протоколов изготовления и оборудования для получения богатой тромбоцитами плазмы. Однако дизайн выполненных исследований, группы пациентов, оценка клинической эффективности, методики применения, аппараты для изготовления во многом различаются. Результаты исследований сложно сортировать и интерпретировать, так как имеется множество протоколов для коммерческих и мануальных методов приготовления богатой тромбоцитами плазмы.
Целью данного обзора явилось выявление наиболее оптимального и обоснованного метода приготовления богатой тромбоцитами плазмы, скорости и времени центрифугирования, антикоагулянта для ингибирования процесса агрегации тромбоцитов и активатора для высвобождения факторов роста.
Выводы: Для получения богатой тромбоцитами плазмы многими используется двойное центрифугирование. Разнообразие скоростей и времени центрифугирования зависит от используемой модели центрифуги и ее центробежной силы. Терапевтический эффект от применения богатой тромбоцитами плазмы достигается при увеличении концентрации тромбоцитов по разным источникам в 2-8 раз, в среднем в 5 раз в зависимости от изначального содержания тромбоцитов в цельной крови. Чаще всего мы наблюдали фигурирование времени в 10 минут и ускорения не ниже 160 g, но и не выше 3000 g. В качестве антикоагулянта чаще встречалось использование цитрата натрия, в качестве активатора помимо раствора хлорида кальция, встречалось неоднократное упоминание о целесообразности использования аутологичного тромбина.
Ключевые слова: богатая тромбоцитами плазма, получение, приготовление